

Human caspase-1 (SEQ ID NO:)
Human caspase-13^a (SEQ ID NO:)
Human caspase-4 (SEQ ID NO:)
Human caspase-5 (SEQ ID NO:)
Human caspase-12 (SEQ ID NO: 4)
Mouse caspase-12 (SEQ ID NO:)
Mouse caspase-11 (SEQ ID NO:)
conserved amino acids^b

Human caspase-1 (SEQ ID NO:)
Human caspase-13^a (SEQ ID NO:)
Human caspase-4 (SEQ ID NO:)
Human caspase-5 (SEQ ID NO:)
Human caspase-12 (SEQ ID NO: 4)
Mouse caspase-12 (SEQ ID NO:)
Mouse caspase-11 (SEQ ID NO:)
conserved amino acids^b

Human caspase-1 (SEQ ID NO:)
Human caspase-13^a (SEQ ID NO:)
Human caspase-4 (SEQ ID NO:)
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Human caspase-12 (SEQ ID NO: 4)
Mouse caspase-12 (SEQ ID NO:)
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conserved amino acids^b

Human caspase-1 (SEQ ID NO:)
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Human caspase-12 (SEQ ID NO: 4)
Mouse caspase-12 (SEQ ID NO:)
Mouse caspase-11 (SEQ ID NO:)
conserved amino acids^b

[illegible]

SCSEGNVVKLSLEEAQRJWKQKSAEIIYPIMDKSSRTRLALIICNEEFDSIPRRRTGAEEVDI
-GSAATLKLCPHEEFFLKCKERAIEIYPIKERKDRLALICIINTEFDHMPPRNGAALDI
-ESTDALKLCPHEEFFRLCKERAEEIYPIKERNNRTRLALIICNTEFHDHLPPRGADFDI
-ESTNILKLCPREEFFRLCKKNHDEIYPIKKREDRRRLALIICNTKFDDLPARNGAHYDI
-----QIYPMKEKRRTCASNIKNKEFNYLHNRNNGSELDL
SEVQDTLKLCPRDQFCIKTERAKEIYYPVMEKEGRTRLALIICNKKFIDYLFDRDNADTDI
--SLNTLKLCSPEEFFRLCREKTQEIYPIKEANGNRTRKALIICNTEFKHLSLRYGAKFDI

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 $\frac{1}{2}$

FIGURE 2

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hCaspase-12	MADEKPSNGV	LVHMKLLIK	TFLDGIFDDL	MENNVLTDE	IHLIGKCLKF
KW-Ap	MADEKPSNGV	LVHMKLLIK	TFLDGIFDDL	MENNVLTDE	IHLIGKCLKF
KW-Bp	MADEKPSNGV	LVHMKLLIK	TFLDGIFDDL	MENNVLTDE	IHLIGKCLKF
KW-Cp	MADEKPSXGV	LVHMKLLIK	TFLDGIFDDL	MENNVLTDE	IHLIGKCLKF
KW-Dp	MADEKPSNGV	LVHMKLLIK	TFLDGIFDDL	MENNVLTDE	IHLIGKCLKF
KW-Ep	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
KW-Fp	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
KW-Gp	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
KW-Hp	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
KW-Ip	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
KW-Jp	MADEKPSNGV	LVHMKLLIK	TFLDGIFDDL	MENNVLTDE	IHLIGKCLKF
KW-Kp	MADEKPSNGV	LVHMKLLIK	TFLDGIFDDL	MENNVLTDE	IHLIGKCLKF
	51				100
hCaspase-12	VVSNAENLVD	DITETAQIAG	KIFREHLWNS	KKQLSSALLE	IQGAQPSGKL
KW-Ap	VVSNAENLVD	DITETAQIAG	KIFREHLWNS	KKQLSS....
KW-Bp	VVSNAENLVD	DITETAQIAG	KIFREHLWNS	KKQLSS....
KW-Cp	VVSNAENLVD	DITETAQIAG	KIFREHLWNS	KKQLSS....
KW-Dp	VVSNAENLVD	DITETAQIAG	KIFREHLWNS	KKQLSS....
KW-Ep	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~PSGKL
KW-Fp	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~AQPSGKL
KW-Gp	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~AQPSGKL
KW-Hp	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~AQPSGKL
KW-Ip	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~AQPSGKL
KW-Jp	VVSNAENLVD	DITETAQIAG	KIFREHLWNS	KKQLSS....
KW-Kp	VVSNAENLVD	DITETAQIAG	KIFREHLWNS	KKQLSSALLE	IQGAQPSGKL
	101				150
hCaspase-12	KLCPHAHFHE	LKTKRADEIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-ApIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-BpIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-CpIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-DpIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-Ep	KLCPHAHFHE	LKTKRADEIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-Fp	KLCPHAHFHE	LKTKRADEIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-Gp	KLCPHAHFHE	LKTKRADEIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-Hp	KLCPHAHFHE	LKTKRADEIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-Ip	KLCPHAHFHE	LKTKRADEIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-JpIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
KW-Kp	KLCPHAHFHE	LKTKRADEIY	PVMEKERRTC	LALNIRNKEF	NYLHNRNGSE
	151				200
hCaspase-12	LDLLGMRDLL	ENLGYSVVIK	ENLTAQEMET	ALRQFAAHPE	HQSSDSTFLV
KW-Ap	LDLLGMRDLL	ENLGYSVVIK	ENLTAQEMET	ALRQFAAHPE	HQSSDSTFLV
KW-Bp	LDLLGMRDLL	ENLGYSVVIK	ENLTAQEMET	ALRQFAAHPE	HQSSDSTFLV
KW-Cp	LDLLGMRDLL	ENLGYSVVIK	ENLTAQ....
KW-Dp	LDLLGMRDLL	ENLGYSVVIK	ENLTAQ....
KW-Ep	LDLLGMRDLL	ENLGYSVVIK	ENLTA....
KW-Fp	LDLLGMRDLL	ENLGYSVVIK	ESLTAQEMET	ALRQFAAHPE	HQSSDSTFLV
KW-Gp	LDLLGMRDLL	ENLGYSVVIK	ENLTAQ....
KW-Hp	LDLLGMRDLL	ENLGYSVVIK	ENLTAQ....
KW-Ip	LDLLGMRDLL	ENLGYSVVIK	ENLTAQEMET	ALRQFAAHPE	HQSSDSTFLA
KW-Jp	LDLLGMRDLL	ENLGYSVVIK	ENLTAQEME.STFLV
KW-Kp	LDLLGMRDLL	ENLGYSVVIK	ENLTAQEMET	ALRQFAAHPE	HQSSDSTFLV

H12_a_~1

CLUSTAL W (1.7) multiple sequence alignment

```
h_Caspase-3 -----
h_Caspase-7 -----
h_Caspase-12 -----MADE
m_Caspase-12 -----MAAR
h_Caspase-4 -----MAEG
h_Caspase-13 -----MAED
h_Caspase-5 -----MFKGILQSGLDNFVINHMLKNNVAGQTSIQTLVPNTDQKSTSVKKD
h_Caspase-1 -----MAD
h_Caspase-6 -----
h_Caspase-8 -----MDFSRNLYDIGEQLDSEDLASLKFLSLDYIPQRKQEPKIDALM
h_Caspase-10 MKSQGQHWYSSSDKNCKVSFREKLLIIDSNLGVQDVENLKFLCIGLVPNKKLEKSSSASD
h_Caspase-9 -----MDE
h_Caspase-2 -----MAADRGRIRILGVCGM
h_Caspase-14 -----
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h_Caspase-3 -----
h_Caspase-7 -----
h_Caspase-12 KPSNGVLVHMVK---LLIKTFLDGI--FDDLMENNVLTDEIHLIGKCL-KFVVSNAEN
m_Caspase-12 RTHERDPIYKIK---GLAKDMLDGV--FDDLVEKNVLNGDELLKIGESA-SFILNKAEN
h_Caspase-4 N-HRKKPLKVLE---SLGKDFLTGV--LDNLVEQNVLNWKKEEEKKKYYD-AKTEDKVRV
h_Caspase-13 K-HNKNPLKMLE---SLGKELISGL--LDDFVEKNVLKLEEEEEKKKIYD-AKLQDKARV
h_Caspase-5 N-HKKKTVKMLE---YLGKDV LHGV--FNYLAKHDVLTLEKEEEKKKYYD-AKIEDKALI
h_Caspase-1 KVLKEKRKLFIR---SMGEGTINGL--LDELLQTRVLNKEEMEKVKREN-ATVMDKTRA
h_Caspase-6 -----
h_Caspase-8 LFQRLQEKRMLEESNLSFLKELLFRINRLDLLITYLNTRKEEMERELQTPGRAQISAYRV
h_Caspase-10 VFEHLLAEDLLSEEDPFFLAELLYIIR-QKKLLQHLNCTKEEVERLLPTR--QRVSLFRN
h_Caspase-9 ADRRLLRRCRLR-----LVEELQVDQLWDALLSSELFPHMIEDIQRAGSGSRDQARQ
h_Caspase-2 HPHHQETLKKNR---VVLAKQLLLSELLEHLLEKDIITLEMRELIQAKV--GSFSQNV
h_Caspase-14 -----
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h_Caspase-3 -----MENTEN-----SVDSK-SIKNLEPKIIH-----GSE-----
h_Caspase-7 -----MADDQGCIEEQGVEDSANED-----SVDAPDRSSFVPSLFS-----KKKKN-----
h_Caspase-12 LVDDITETAQIAGKIFREHLWNS-----KKQLSSALL--EIQGAQ-----PSG-----K-----
m_Caspase-12 LVENFLEKTD MAGKIFAGHIANS-----QEQLSLQFSNDEDDGPQKICTPSSPSES KRKV
h_Caspase-4 MADSMQEKQRMAGQMLLQTFNID-----QISPNKKAHPNMEAGPP-----ESGES-----
h_Caspase-13 LVDSIRQKNQEAGQVFVQTFNLNID-----KNSTSIKAPETVAGPD-----ESVGS-----
h_Caspase-5 LVDSL R-KNRVAHQMFQTQTLNMD-----QKITSVKPLLQIEAGPP-----ESAES-----
h_Caspase-1 LIDSVIPKGAQACQICITYICEEDS---YLAGTLGLSADQTS GNYLNMQDSQGVLS SFPA
h_Caspase-6 -----MSSASGLRRGHPAGGE-----EN-----
h_Caspase-8 MLYQISEEVSRSSELRSFKLLQEEISKCKLDDDMNLLDIFIEMEKRVILGEGKLDILKRV
h_Caspase-10 LLYELSEGIDSENKDMIFLLKDSLP-KTEMTSLSF LAFLEKQ GK---IDEDNLTCLEDL
h_Caspase-9 LIIDLETRGSQALPLFISCLEDTG---QDMLASFLRTNRQA AKLSKPTLENLTPVVL RP
h_Caspase-2 LLNLLPKRGPQAFDAFCEALRETKQGHLEDMLLTTLSGLQHVLPPLSCDYDL SL PFPVCE
h_Caspase-14 -----
```

FIGURE 4

h_Caspase-3	FESFSFDATFHAKKQIPCIVSMLTKE--LYFYH----
h_Caspase-7	FESQSDDPHFHEKKQIPCVVSMLTKE--LYFSQ----
h_Caspase-12	FET---- PNILTQLPTIERLSMTRYF--YLFPGN---
m_Caspase-12	FEV----PGELTQMPTIERVSMTRYF--YLFPGN---
h_Caspase-4	FET----PRAKAQMPTIERLSMTRYF--YLFPGN---
h_Caspase-13	FEK----PNVKAQMPTVERLSMTRYF--YLFPGN---
h_Caspase-5	FEV----PQAKAQMPTIERATLTRDF--YLFPGN---
h_Caspase-1	FEQ----PDGRAQMPTTERTVTLTRCF--YLFPGH---
h_Caspase-6	RVDFCKDPSAIGKKQVPCFASMLTKK--LHFFPKSN-
h_Caspase-8	VSN--KDDKKNMGKQMPQPTFTLRKK--LVFPPSD---
h_Caspase-10	KRTVWG-AKQISATSLPTAISAQTPRPPMRRWSSVS-
h_Caspase-9	VSV-----KGIYKQMPGCFNFLRKK--LFFKTS---
h_Caspase-2	REGYAPGTEFHRCKEMSEYCSLTCRH-LYLFPGHPPT
h_Caspase-14	MAEAELVQEGKARKTNPEIQSTLRKR--LYLQ-----

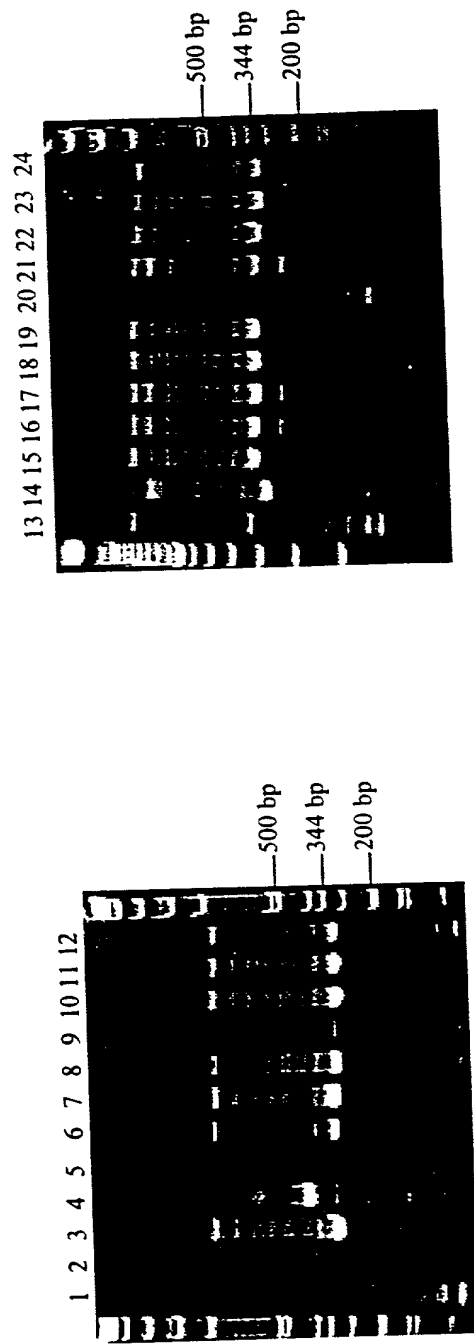
Legend:

- ↓ Active-site Residues
- * Identical Residues
- : Conservative Substitution
- . Allowable Substitution

CARD domain ICE-P20 Domain ICE-P10 Domain Active-site Residues

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Tissue Profiling of hCaspase-12



1. Brain 2. Heart 3. Kidney 4. Spleen 5. Liver 6. Colon 7. Lung 8. Small Intestine
9. Muscle 10. Stomach 11. Testis 12. Placenta 13. Pituitary 14. Thyroid gland
15. Adrenal gland 16. Pancreas 17. Ovary 18. Uterus 19. Prostate 20. PBL 21. Fetal brain 22. Fetal liver 23. Fat 24. Mammary gland

FIGURE 6

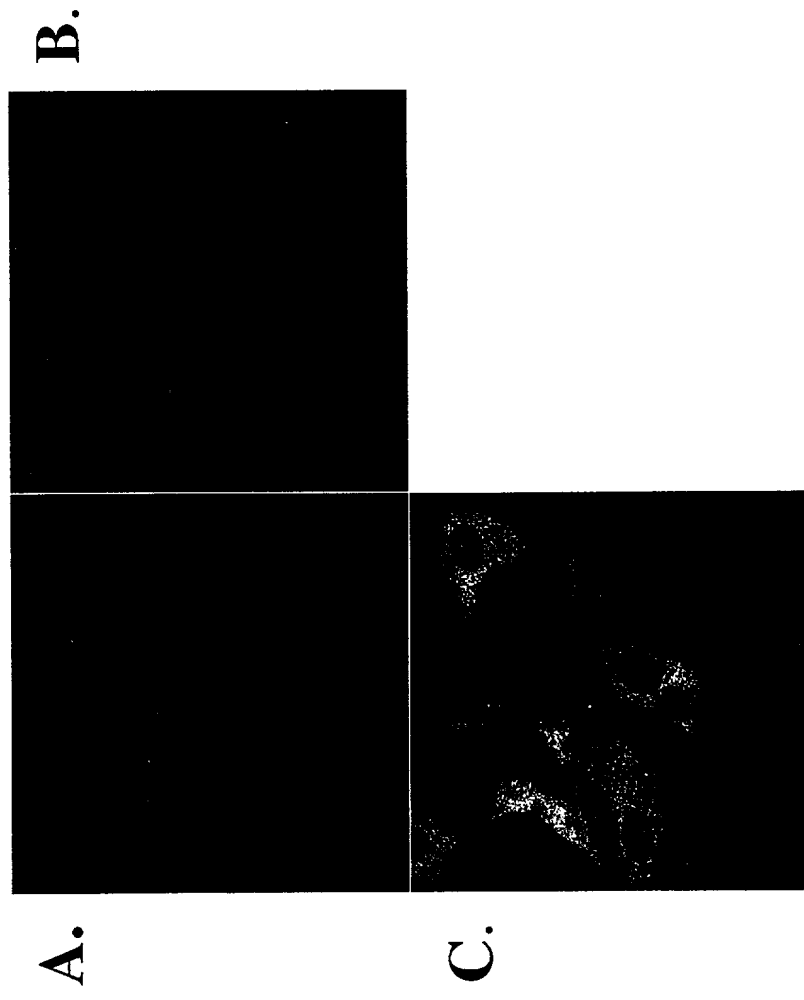


FIGURE 7

SH-EP cell transfection +/- α -Fas

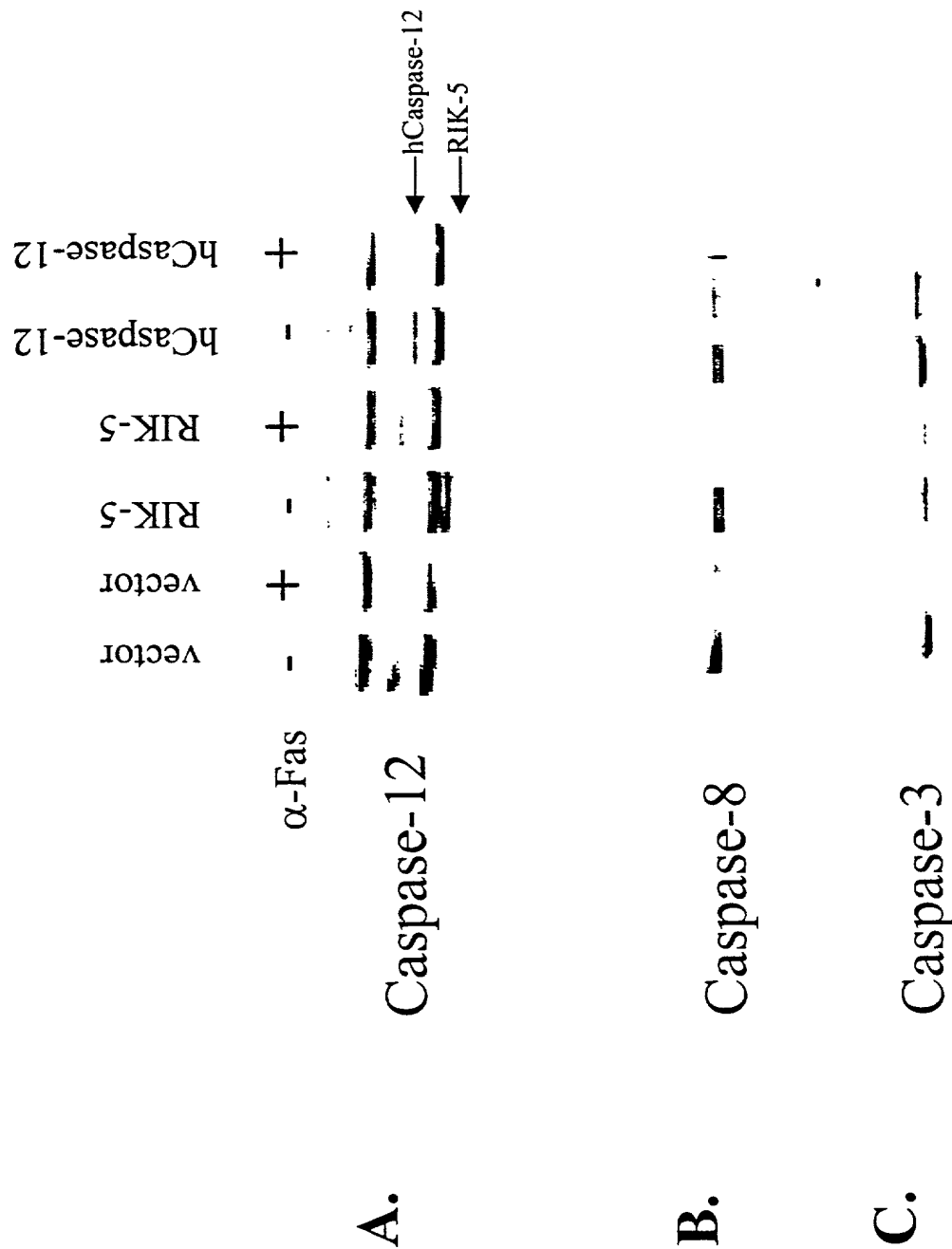


FIGURE 9

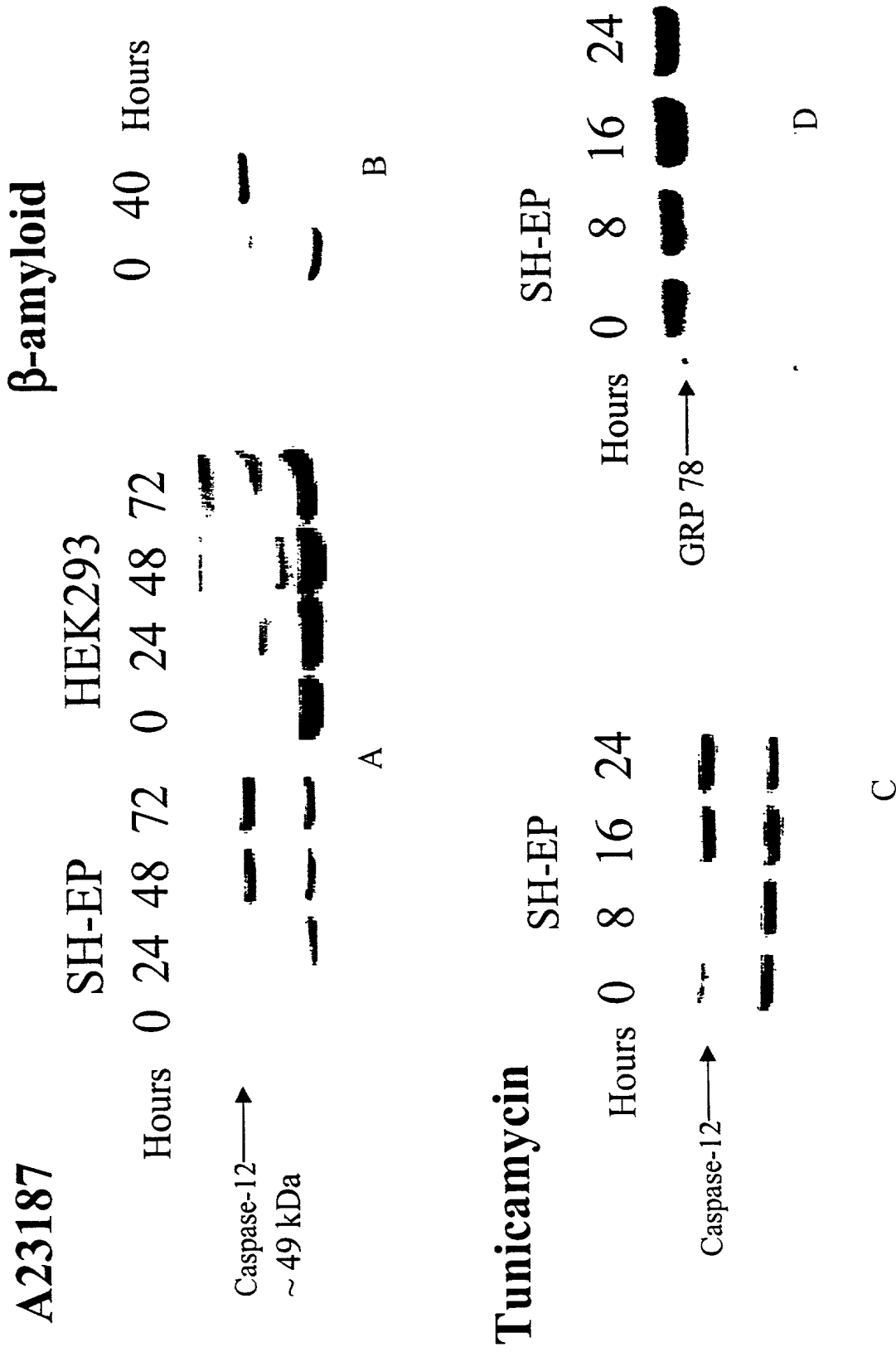


FIGURE 10

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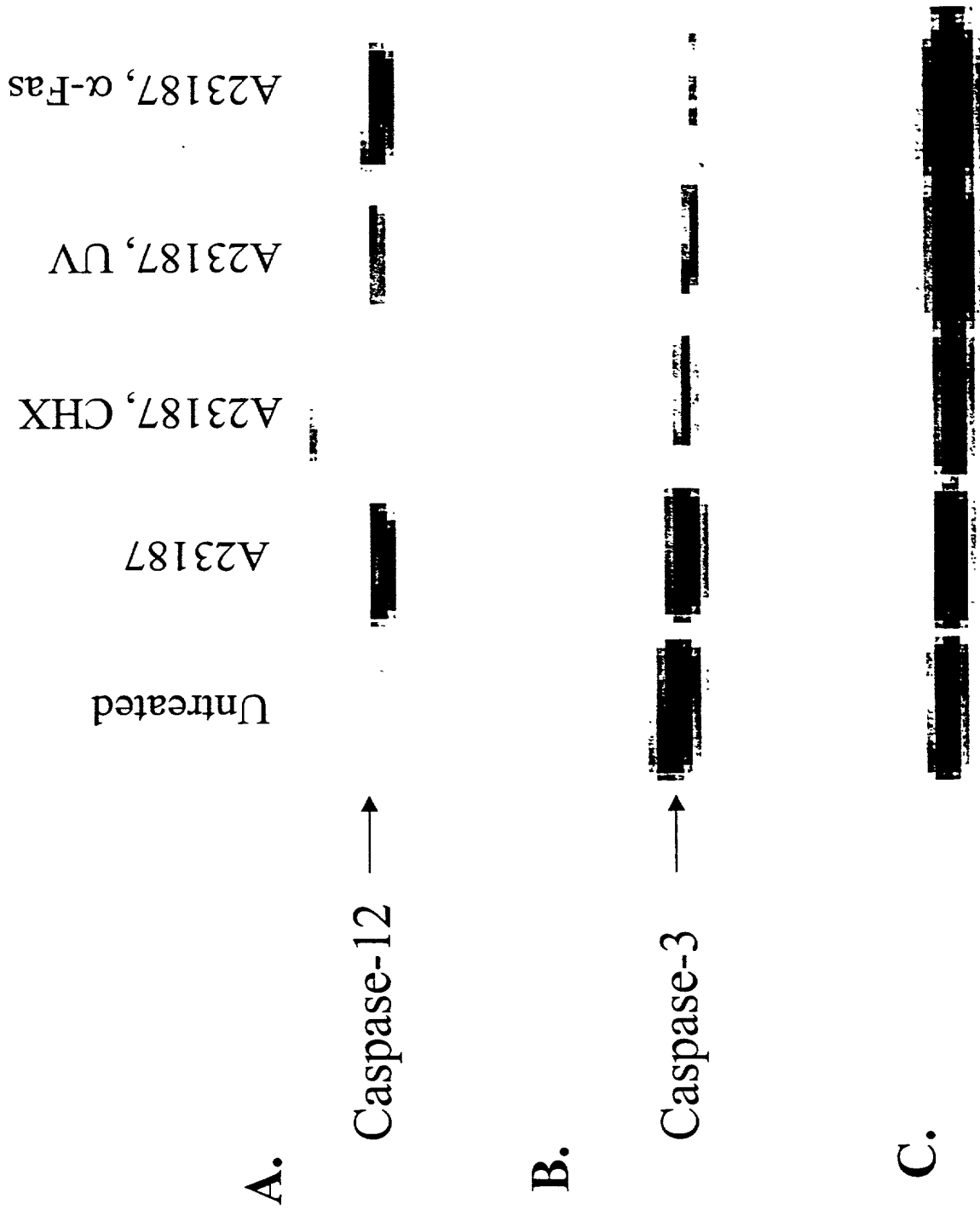


FIGURE 11

SH-EP cells treated with A23187, UV +/- inhibitors



FIGURE 12

Calpain cleavage of recombinant caspase-12

